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Alteration of the Reproductive Cycle of Female Wistar Rat *via* UVB Induced Hyperthyroidism and Therapeutic Effect of Curcumin and Ascorbic Acid

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Abstract: **The present study therapeutic effects of curcumin and ascorbic acid against the alteration of the reproductive cycle *via* UVB-induced hyperthyroidism in female Wistar rats. Thirty-six female Wistar rats sexually matured older weight 130-150 g and aged 12-16 weeks had arbitrarily divided into six groups. The first group was a control group, which received standard food and water ad libitum. The second UVB group was exposed to a dose of 280 nm to UVB radiation for two hours daily. The third UVB+Curcumin group received 280 nm of UVB radiation for two hours daily and an oral dose of curcumin (25 mg/kg body weight) daily. The fourth UVB+Ascorbic acid group received 280 nm UVB radiation for two hours daily and an oral dose of ascorbic acid (250 mg/kg body weight) daily. The fifth, curcumin groups (25 mg/kg body weight), and the sixth is ascorbic acid groups (250 mg/kg body weight). All the treatments last for 15 consecutive days. UVB-induced hyperthyroidism caused structural alteration of the estrous cycle in the female Wistar rat compared to the control group. Curcumin and ascorbic acid prevent the estrous phases and their morphology.**

Index Terms: **Ascorbic acid, Curcumin, Hyperthyroidism, Reproductive cycle, Wistar rat**

I. INTRODUCTION

Ultraviolet B (UVB) radiation is the medium wavelength (280-320 nm) electromagnetic radiation and reaches the earth's surface due to ozone depletion and affects the biological system (Rai et al., 2018). Electromagnetic radiation has been used considerably in many resources such as mercury lamps, dental polymerizing devices, X-rays devices, blacklight lamps, welding systems, counterfeit money detectors, and so forth. Radiation influences the body organs such as the thyroid gland (Walters et al., 1930 and Esmekaya et al., 2010), eyes (Balci et al., 2009), liver (Mahobiya 2020), pores, and skin (Matsumura et al., 2004). Previously reported radiation causes thyroid dysfunction with various structural, functional, and behavioral changes, with reproductive illnesses in females (Choksi et al., 2003).

The reproductive cycle in mammals is known as the estrous cycle and the best exception of primates, which have menstrual cycles. The estrous cycle of non-primate vertebrates consisting of mice, rats, horses, and so on represented the cyclic pattern of the ovarian action that allowed females to go from a duration of reproductive receptivity to non-receptivity, subsequently leading to being pregnancy after successful mating. In rodents' inclusive rats, the estrous occurred every 4-5 days, with sequential stages of proestrus, estrus, metaestrus, and diestrus for 1, 1, 1, and two days, respectively (Mclean et al., 2012). These stages occurred in each cycle and in a sequential way. The phases of the estrous period were the first-rate decided by way of the cellular kinds discovered within the vaginal smear. In estrous cycles, females are only sexually energetic during the estrus phase. Animals have this cycle reabsorb the endometrium if conception does not arise throughout the estrous period. On the other hand, animals' menstrual cycles shed the endometrium through menstruation instead. The estrous period is from the onset of estrus until the upcoming estrus. The length of the estrous period varies depending on the animal species. The average length of the estrous cycle is 4–5 days in rats, but it is highly variable. The estrus stage signifies a period when females show signs of mating behavior. Rat spontaneously ovulates during each estrous cycle. Females become cyclic when they reach puberty by four weeks (Rai et al., 2020). The different phases of the estrous cycle in mature females have regulated the mode of a functional hypothalamic- pituitary–ovarian axis.

Thyroid hormones are vital for the appropriate functioning of the female reproductive system since they modulate the metabolism and development of ovarian, uterine, and placental tissues. Consequently, hypothyroidism and hyperthyroidism may result in

subfertility or infertility in females. Other properly-documented sequelae of maternal thyroid dysfunctions contain menstrual/estrous irregularity, anovulation, abortion, preterm transfer, preeclampsia, intrauterine progress limit, postpartum thyroiditis, and psychological retardation in children. (Silva et al., 2018). Both hypo- and hyper-thyroidism had been associated with an altered ovarian characteristic, menstrual irregularities, subfertility, and increased miscarriage rates (Krassas et al., 2010; Van den Boogaard et al., 2011), recommended that thyroid hormone affects female reproductive organs. Thyroid hormone receptors and transporters are expressed in almost all reproductive and non-reproductive cells of both the male and female gonads, even as a large number of thyroid-dependent molecules are present inside the male and female gonads (Colicchia et al., 2014). In the previous studies, UVB exposure exhibited significantly increased T3 and T4 and significantly decreased TSH levels (Rai G et al., 2020). The thyroid hormone influences human reproduction via different mechanisms at both the central and the peripheral levels. In men and females, hyperthyroidism may lead to infertility, that resolved as soon as a euthyroid state is accomplished (Mintziori et al., 2016).

To the best of our knowledge, the present study is innovative in its field, the effect of UVB-induced hyperthyroidism on the reproductive cycle of female Wistar rats. In the present study, we investigated the therapeutic effect of antioxidants against the morphological changes in cells induced by UVB irradiated hyperthyroidism.

II. Materials and Methods

A. Chemicals

All the chemicals and reagents used were of analytical grade. We purchased ascorbic acid and curcumin from Sigma-Aldrich Co., the USA, and Himedia, India, respectively, and crystal violet and the remained chemicals from Central Drug House (P) Ltd, New Delhi, India.

B. Ethics statement

Department of Pharmaceutical Sciences Dr. Harisingh Gour Vishwavidyalaya (A Central University) Sagar (M.P.), India (Ethical Registration379/Go/ReBi/S/01/CPCSEA) approved this study and followed international guidelines for the care and use of laboratory animals.

C. Experimental animals and treatment

130-150 gm female rats aged 12-16 weeks bought from the College of Veterinary Sciences and Animal Husbandry Mhow, India. All animals (n=36) were housed in plastic cages and fed on standard laboratory diet daily food and water ad libitum. Rats kept on laboratory conditions, with optimum temperature (20±2), relative humidity (40%–60%), and 12h/12h light and dark cycle. Distribution of animals into six groups of 6 animals each group did randomly. The control group received food and water ad libitum. The UVB group received a dose of 280 nm UVB radiation for two hours daily for 15 days. The UVB+Cur group received 280 nm UVB radiation for two hours daily and an oral dose of curcumin (25 mg/kg body weight) daily for 15 days. The UVB+AA group received 280 nm UVB radiation for two hours daily and an oral dose of ascorbic acid (250 mg/kg body weight) daily for 15 days. The fifth group is curcumin (25 mg/kg body weight), and the sixth group is ascorbic acid (250 mg/kg body weight).

D. Collection of vaginal smears

At the start of the experiment, sexually mature female animals were exposed dose of 280 nm of UVB radiation for two hours daily for 15 days. From the seventh to fifteen days of exposure, collected vaginal smear by using a sterilized micropipette. The micropipette was filled with a small amount of double-distilled water and inserted into the vagina of the female rat. Vagina was flushed two to three times with the double-distilled water then the fluid was placed onto a glass slide. Vaginal smear spread onto the glass slides, and stained with crystal violet (1%) and observed under a light microscope with 10× and 40× magnification (Carl Zeiss, Germany) (Yener et al., 2005).

E. Bodyweight

At the start of the experiment measured body weight from five days intervals were done with an electronic machine (Sartorius, BP210 S).

F. Serum collection

Last, of the experiment, animals were anesthetized and collected blood through cardiac arrest. Blood stands for room temperature, then collect serum and stored at -20 ºC for hormones estimation and measurement of T3, T4, TSH, FT3, and FT4 using an ELISA kit provided by The Calbiotech Inc. (California, USA) (Sachidhanandam et al., 2010).

G. Statistical analysis

All statistical data analyses were using one-way ANOVA. The values expressed as mean ± SE. Dunnet t-test was applied for comparison between control and each treated group individually. Level of significance at *$p<0.05$, **$p<0.01$ and ***$p<0.001$ was considered significant.

III. Results

A. Bodyweight

The UVB exposed group showed a significant reduction in net body weight compared to the control group (*$p<0.01$). Co-administration of curcumin and ascorbic acid in UVB+Cur and UVB+AA groups, the net body weight was significantly increased ($p<0.01$) in comparison to the UVB exposed group, and significant differences were observed in Cur and AA administrated group when compared with the control group. **(Table I)**

B. Thyroid hormones

Thyroid hormones imbalance showed hyperthyroidism. T3, T4 (**$p<0.01$, ***$p<0.001$), FT3, and FT4 levels (*$p<0.05$) significantly increased in UVB exposed female Wistar rats as compared to the control group. Co-administration of curcumin and ascorbic acid in the UVB exposed female rats significantly

decreased T3, T4, FT3, and FT4 levels and decreased significantly (***p<0.001) in TSH level in UVB exposed female Wistar rats. No changes showed on curcumin and ascorbic acid administrative female Wistar rats compared to the control group. **(Table I)**

C. Determination of the estrous cycle

In this experiment, we observed a UVB-induced hyperthyroidism condition that leads to the alteration and irregularities in estrous cycle phases.

1) Proestrus phase: -

The presence of nucleated epithelial cells indicates the proestrus phase. The Control group showed normal nucleated epithelial cells. UVB-induced hyperthyroidism group showed nucleated epithelial cells in a cohesive cluster and damaged. UVB+Cur and UVB+AA groups showed repair and separation of nucleated epithelial cells. Curcumin and ascorbic acid groups showed normal nucleated epithelial cells compared to the control group **(Fig 1).**

2) Estrus phase: -

In the estrus phase, cornified squamous epithelial cells were observed uniformly in the control group. UVB-induced hyperthyroidism group had shown damaged cornified squamous epithelial cells. UVB+Cur and UVB+AA groups showed repaired the cornified squamous epithelial cells. Curcumin and the ascorbic acid group showed cornified squamous epithelial cells the same as a control group **(Fig 2).**

Table I. Changes in serum thyroid hormone concentration and alteration in body weight

Groups	T3	T4	TSH	FT3	FT4	Body weight
Control	38.8±0.454	3.1±0.082	1.0±0.007	1.83±0.099	0.807±0.15	120.0±0.54
UVB treated	67.2±0.530***	3.6±0.075**	0.012±0.003***	2.64±0.018**	0.96±0.005**	87.6±1.21***
UVB+Cur	45.2±0.562***	3.0±0.075NS	0.3±0.006***	1.64±0.024**	0.82±0.0136NS	95.1±2.82**
UVB+AA	52.5±0.771***	3.5±0.073**	0.02±0.006***	2.17±0.043**	0.912±0.119**	98.6±2.67**
Curcumin	38.75±1.19NS	3.17±0.151NS	0.992±0.011*	1.765±0.068NS	0.822±0.042NS	127.91±1.65**
Ascorbic acid	38.5±0.745NS	3.15±0.067NS	0.99±0.008*	1.9125±0.033NS	0.8±0.0194NS	129.33±1.45**

3) Metestrus phase: -

In the metestrus phase, the control group showed normal squamous cornified epithelial cells with an equal proportion of leukocytes. UVB-induced hyperthyroidism group showed changes of cornified cells and reduced the leukocytes cells. UVB+Cur and UVB+AA groups were showed prevention of cornified and leukocyte cells. Curcumin and ascorbic acid groups were showed no changes in cornified and leukocytes cells compared to the control group. **(Fig 3).**

4) Diestrus phase: -

In the diestrus phase, the control group showed normal leukocytes cells. UVB-induced hyperthyroidism group showed distorted leukocytes cells. UVB+Cur and UVB+AA groups showed the prevention of leukocytes cells. No changes showed in curcumin and ascorbic acid groups compared to the control group. **(Fig 4).**

DISCUSSION

In the present investigation, the physiological changes in the reproductive cycle of female Wistar rats after UVB-induced hyperthyroidism. Thyroid hormones are linked with steroid hormones receptor and affect ovarian function. Due to hypothyroidism is failed ovulation, and hyperthyroidism generates ovarian cancer (Silva et al., 2018).

In previous studies, the reproductive system influences the estrous cycle on non-reproductive function (Marques et al., 2017; Vanderlei et al., 1996; Spadari-Bratfisch et al., 1999). Veginal smear cytology characterized each phase and their cells as epithelial cells, cornified cells, and leukocytes (Hoar et al., 1975). In result showed that the UVB exposure increased T3, T4 and decreased TSH level and body weight significantly, indicating hyperthyroidism. The UVB-induced hyperthyroidism showed a negative effect on the estrous cycle of female Wistar rats. Due to hyperthyroidism, cohesive cluster forms of nucleated epithelial cells of the proestrus phase were seen (Figure 1). In estrus phase damaged the cornified cells (Figure 2), and the metestrus phase showed some changes in cornified cells and reduced the number of leukocytes cells (Figure 3). The diestrus phase showed distorted leukocytes cells due to UVB-induced hyperthyroidism (Figure 4). Curcumin and ascorbic acid cured the estrous cycle and showed a positive effect in the estrous phases in the UVB-induced hyperthyroidism group. Previously investigated thyroid hormones affect physical and ovarian progress, the days of the vaginal opening, the number of corpora lutea, and reproductive hormone production in female rats, probably acting through the NOS signaling pathway to alter estrous cyclicity (Wei, et al., 2018). Thyroid hormones dysfunction changes reproductive hormonal profiles and delays the beginning of the estrous cycle (Liu et al., 2018).

Figure 1

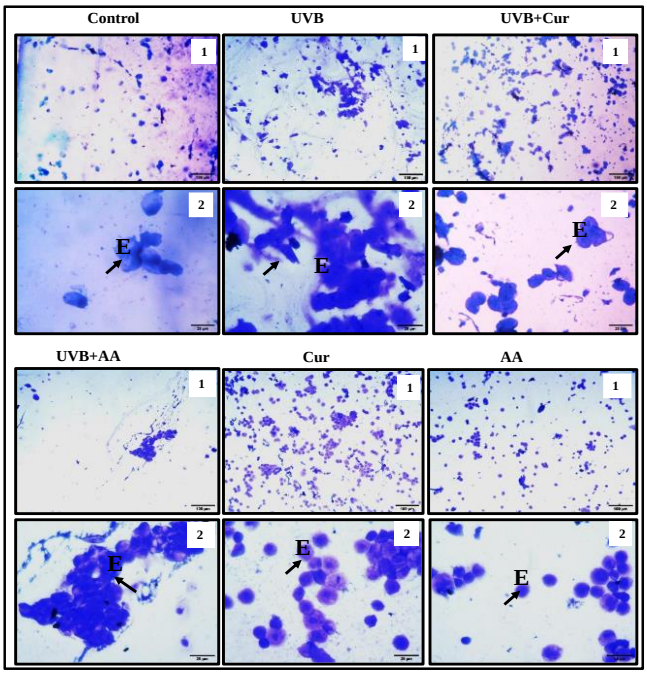

Figure 1: Photograph showing proestrus phase. E represents epithelial cells. The Control group showed normal nucleated epithelial cells, the UVB group showed cluster form of nucleated epithelial cells, UVB+Cur and UVB+AA group showed repair and separation of nucleated epithelial cells, Cur and AA groups showed normal nucleated epithelial cells. 1 and 2 represent 100X and 400X

Figure 2

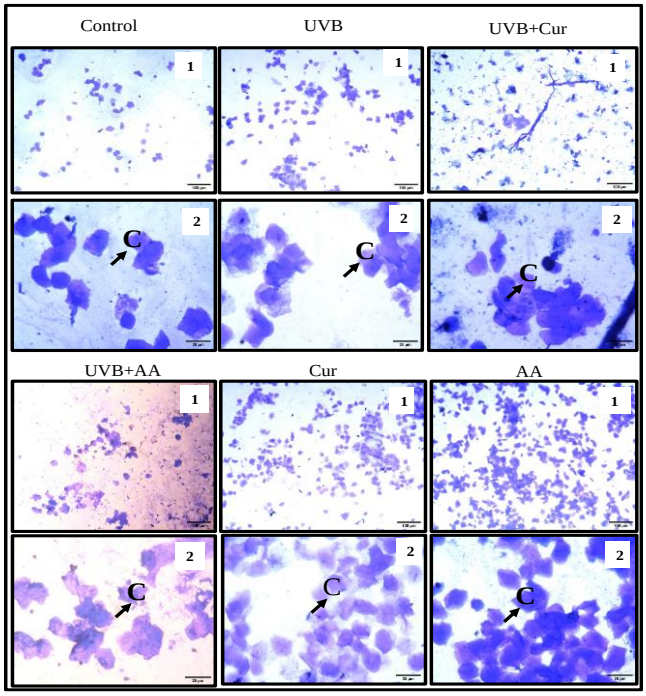

Figure 2: Photograph showing estrus phase. C represents cornified cells. The Control group showed normal cornified cells, UVB group showed damaged cornified squamous epithelial cells. UVB+Cur and UVB+AA groups showed repaired cornified squamous epithelial cells. Cur and the AA group showed cornified squamous epithelial cells.
1 and 2 represent 100X and 400X magnification.

Figure 3

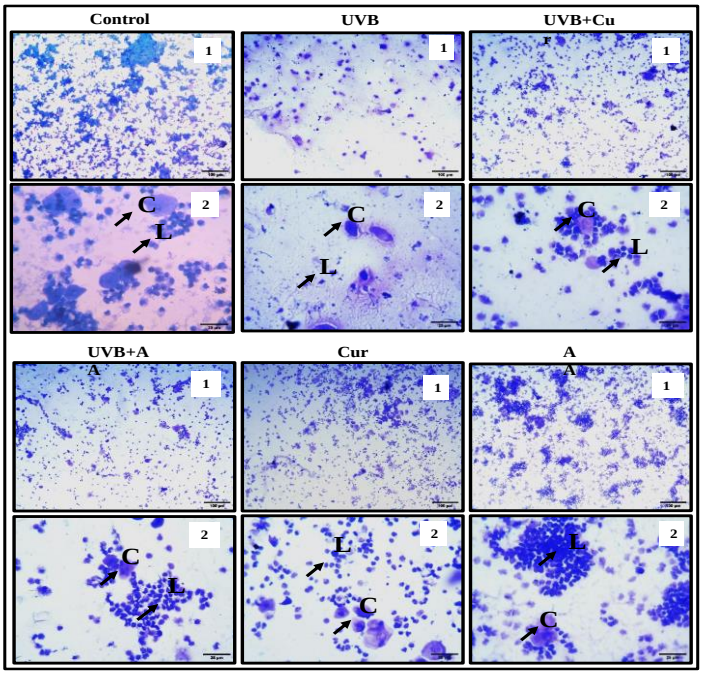

Figure 3: Photograph showing metestrus phase. C represents cornified cells and L, leukocytes cells. The Control group showed cornified epithelial cells with leukocytes at equal proportion, UVB group distorted leukocytes cells. UVB+Cur and UVB+AA groups showed prevention of leukocyte cells. Cur and AA groups showed normal leukocytes cells. 1 and 2 represent 100X and 400X magnification.

Figure 4

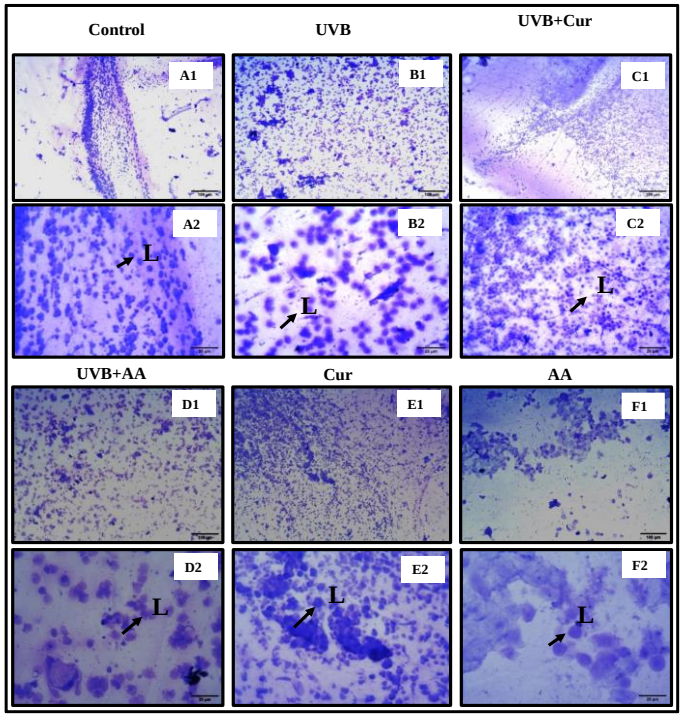

Figure 4: Photograph showing diestrus phase. L represents leukocytes cells. The Control group showed normal leukocytes cells, UVB group showed distorted leukocytes cells. UVB+Cur and UVB+AA groups showed the prevention of leukocytes cells. Cur and AA groups showed

In a previous study, the effect of thyroid hormones on fertility and fetal development has been extensively investigated by assessing adverse outcomes in individuals with thyroid dysfunction in female Wistar rats. Thyroid hormones influence molecular mechanism that affects estrous cycle control, sexual maturation and behavior, ovulation, maternal ability, pregnancy maintenance, postnatal, fetal growth, and lactation (Freitas et al., 2007, Silva JF et al., 2014, Leite et al., 2008). In hyperthyroidism, increased sex hormones bind globulin, increase the total circulating steroid levels with a reduced free fraction (Redmond 2004). The overactive thyroid hormones in females before puberty cause delayed menstruation or amenorrhea (Krassas et al., 2010). T3 hormone must be present at the end of the breeding to start the estrous cycle (Vasudevan et al., 2002). Hypothalamus releasing GnRH alters the number of circulating gonadotropins, FSH, and LH from the pituitary lead to sexual maturity. While raised levels of FSH trigger follicular growth and maturation, ovulation occurs under the influence of increasing levels of LH. These changes showed in ovarian steroid production during each cycle. Curcumin and ascorbic acid showed the therapeutic effect of the endocrine and reproductive system, decreased T3 and T4 hormones as well as brought an increase in TSH hormones. In the present study dose of curcumin and ascorbic acid used (25 mg/kg and 250 mg/kg) showed a therapeutic effect on different phases of the estrous cycle in female Wistar rats (Rai G et al., 2020). However, higher dose concentration of curcumin and ascorbic acid-induced the blockage of estrous phases and showed significant antifertility activity and elevated estrogenic activity, with inhibition of ovulation and impairment of fertility (Thakur et al., 2009).

CONCLUSION

It concluded that UVB radiation is a high-up environmental toxin that reaches the earth's surface due to ozone depletion and affects the biological system. UVB radiation disturbs the endocrine system and causes thyroid toxicity. UVB-induced hyperthyroidism causes a significant disturbance and leads to irregularity in the estrous period and their cells. Both curcumin and ascorbic acid protect the estrous phases and their cells.

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